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Aldicarb and Related Compounds in Drinking Water Via Direct Aqueous Injection HPLC (High Performance Liquid Chromatography) with Post Column Derivatization

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ALDICARB AND RELATED COMPOUNDS IN DRINKING WATER VIA DIRECT AQUEOUS INJECTION HPLC WITH POST COLUMN DERIVATIZATION

by

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16. ABSTRACT

The U.S. Environmental Protection Agency is currently performing a statewide monitoring survey for Temik (aldicarb) in the drinking water supplies of Florida. Prior to beginning this study, the Environmental Monitoring and Support Laboratory in Cincinnati, Ohio developed and validated the analytical procedure to be used in this study. After filtration, the water sample is directly injected into an HPLC column. After eluting from the column, the pesticide is hydrolyzed with sodium hydroxide and derivatized with o-phthaldehyde to form a fluorescent derivative. This procedure, which involves practically no sample work-up, is applicable to concentrations from below 10 µg/L to well over 500 µg/L.

The procedure has been validated for aldicarb, aldicarb sulfone, aldicarb sulfoxide, oxamyl, methomyl, 3-hydroxy carbofuran, carbofuran, and carbaryl. The results of method detection limit studies and sample storage studies are presented.

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ALDICARB AND RELATED COMPOUNDS IN DRINKING WATER VIA DIRECT AQUEOUS INJECTION HPLC WITH POST COLUMN DERIVATIZATION

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INTRODUCTION

In the fall of 1983, the U.S. Environmental Protection Agency (USEPA) was charged by the Congress of the United States to conduct a monitoring study which would evaluate the degree of and potential for aldicarb contamination in the drinking water supplies of the state of Florida. The Agency was to place special attention to water supplies proximate to citrus groves which had a history of aldicarb application. The results of this study are to be presented to Congress in January 1985.

Prior to beginning this study, the Environmental Monitoring and Support Laboratory in Cincinnati was assigned the task of developing the analytical procedure to be used in this study. The analytical procedure was expected to achieve the following objectives:

1) aldicarb, its sulfone and sulfoxide would each be measured;
2) the detection limits would be in the range of 1 µg/L;
3) sample processing would be minimal; and,
4) the procedure would be amenable to unattended operation.

Although analytical procedures for aldicarb were available, they did not fully achieve Objectives 1 and 3. Typical procedures called for the addition of peracetic acid to the aqueous sample prior to extraction with methylene chloride. The oxidizing acid converts aldicarb and aldicarb sulfoxide to aldicarb sulfone. The conversion to the sulfone was necessary to produce a single peak in the GC chromatogram. All three analytes undergo

pyrolytic cleavage in the injection port of a gas chromatograph and aldicarb sulfoxide gives multiple peaks after pyrolysis. A flame photometric detector operating in the sulfur mode was used to give selectivity. This approach does not achieve Objective 1 in that only total aldicarb residues are reported.

Reverse phase High Performance Liquid Chromatography (HPLC) conditions had been demonstrated (1) that would permit achieving Objective 1. The technique of post column fluorogenic labeling (2) for these carbamoyl and carbamoyloxime analytes provides an extremely sensitive detection system which could allow achieving Objectives 2 and 3 if direct aqueous injection were used rather than solvent extraction of the aqueous sample. The bulk of methods development involved validating an HPLC procedure which used the technique of post column fluorogenic labeling and achieved Objectives 3 and 4.

EXPERIMENTAL SECTION

Liquid Chromatograph A Waters HPLC system equipped with a model 720 Programmable System Controller, a model WISP 710B autosampler, a model 730 Data Module, and three model 6000A pumps were used as the basic liquid chromatograph. All injections were performed using the autosampler injection system which is selectable between single- and multi-sample modes of operation.

Post Column Derivatization Equipment A Kratos model URS-051 Post Column Reaction System equipped with dual pumps, dual vortex mixers and dual 1.0 mL reaction coils was used to manipulate the post column reagents and mix them into the effluent from the HPLC column. A Kratos model URA-200 HPLC Temperature Controller was used to heat and maintain the first delay coil at 95°C.

Post Column Reagents The derivatization reagents were prepared fresh daily. The 0.05 N sodium hydroxide reagent was prepared by dissolving 2.0 g of sodium hydroxide (ACS Reagent grade) in reagent water and diluting to 1.0 L with reagent water. The o-phthalaldehyde (OPA) reagent was prepared using a multi-step procedure. First, a stock solution of 2-mercaptoethanol (1+1) in acetonitrile was prepared and used throughout the study. Second, the 0.05 N sodium borate buffer was prepared 24 hours in advance by placing 19.1 g of sodium borate decahydrate (ACS Reagent grade) in a volumetric flask and diluting to 1.0 L with reagent water; this assured that all the sodium borate would be dissolved when the solution was filtered. Third, at the beginning of each day, 100 mg of OPA (mp 55 to 59°C) was dissolved in 10.0 mL of methanol and added to the sodium borate buffer. The OPA solution and the sodium hydroxide solution were each filtered (0.05 µm) and degassed for 1 minute using a 200 mL/m stream of helium. After degassing the OPA solution, 100 µL of the 2-mercaptoethanol (1+1) solution was added, the bottle was capped and shaken for 30 seconds. Adding the 2-mercaptoethanol after degassing minimizes losses of this volatile reagent.

Detectors A Kratos model 970 fluorometer equipped with dispersive optics for excitation and cut-off filters for emission was used as the fluorescence detector. Optimum settings were 235 nm for excitation, >419 nm for emission, and a time constant of 2 seconds. A Bioanalytical System model LC-2A detector equipped with a glassy carbon electrode was used during the evaluation of electrochemical detectors. All potential measurements are versus a standard Ag/AgCl reference electrode.

HPLC Columns All columns were commercially available from Waters, Supelco, Dupont, and EM Reagents. A full description of the individual columns is found in Table 1.

HPLC Mobile Phase Methanol and acetonitrile were filtered (0.5 μm) and degassed daily. Water (Milli Q) was also filtered (0.45 μm) and degassed daily.

Standards Authentic reference standards were obtained from the Pesticide and Industrial Chemicals Repository, USEPA, Research Triangle Park, North Carolina 27711. Stock standards in acetonitrile were prepared at a nominal value of 1 mg/mL. Additionally, concentrated solutions of aldicarb, aldicarb sulfoxide, and aldicarb sulfone were obtained from the Quality Assurance Branch, Environmental Monitoring and Support Laboratory (EMSL), USEPA, Cincinnati, Ohio 45268.

RESULTS AND DISCUSSION

Chromatography Several HPLC columns were evaluated in an effort to find a column which maximized the resolution for aldicarb sulfoxide and aldicarb sulfone. Resolution was measured using the following equation.

$$R = \frac{1}{4} n \frac{x-1}{\alpha} \frac{k}{k+1}$$

where:
n = plates per meter,
α = (tb-to)/(ta-to) for tb > ta, and
k = the capacity factor

This evaluation also examined the effect of solvent interactions upon resolution. The mobile phase was varied in its composition of methanol, acetonitrile, and water. Resolution under isocratic conditions and gradient conditions was also examined. Figure 1 summarizes the mobile phase compositions used during the evaluation of each HPLC column. Figure 1-a is a ternary diagram of the mobile phase compositions used under isocratic conditions. The resolution of the sulfoxide and sulfone was measured at 10 different compositions of mobile phase. (Composition 1 was 50/50/0 acetonitrile, water, methanol, composition 5 was 10/80/10 acetonitrile, water methanol, etc.). Figure 1-b summarizes the linear gradients used under gradient conditions. Gradient A was a linear gradient from 100 %

water to 100 % acetonitrile, and Gradient C was a linear gradient from 100% water to 50%/50% acetonitrile, methanol. Gradients A, C, and E were used on all 11 HPLC columns. Gradient B and D were also used with some of the HPLC columns.

Under isocratic conditions, all columns except the cyano (CN) and trimethylsilyl (TMS) gave results similar to those shown in Figure 2. In general, the resolution between aldicarb sulfoxide and aldicarb sulfone was markedly better for a given composition of acetonitrile/water than for the same composition of methanol/water. The cyano column never coeluted the sulfoxide and sulfone (R = 0.59 at 50/50 methanol/water) under the conditions used, however the maximum resolution (R = 2.35 at 20/80 acetonitrile/water) was less than half the maximum resolution of the Rad Pak C-18 8 mm ID column. The TMS column exhibited a unique phenomenon. When the mobile phase composition was changed from 20% acetonitrile/80% water to 20% methanol/80% water, the sulfoxide and sulfone reversed their order of elution. This phenomenon is useful for confirmatory analyses. The presence of the sulfoxide and sulfone can be confirmed if the suspect peaks change elution order when the organic phase is changed from pure acetonitrile to pure methanol.

Under gradient conditions, all columns gave results paralleling those obtained under isocratic conditions. The cyano column (Figure 3) displayed little change in resolution with respect to the final composition of the organic phase. The TMS column (Figure 4) reversed the elution order of the sulfoxide and sulfone when Gradient E was used. Both PAH columns, the C-8 and RP-2 columns maintained resolution under all gradients used. The remaining columns gave adequate resolution for gradients A (to 100% acetonitrile) and C (to 50%/50% acetontrile/methanol), but coeluted the sulfoxide and sulfone under gradient E (to 100% methanol).

The Rad-Pak C-18, 8 mm ID HPLC column was selected for all subsequent work because it gave the maximum resolution under gradient conditions (R = 5.1 for Gradient A). The target analytes for the procedure were later expanded to include oxamyl, methomyl, 3-hydroxycarbofuran, carbofuran, and carbaryl. At first this presented a problem because oxamyl and aldicarb sulfone coeluted under the conditions of Gradient A on this Rad Pak column. Rather than repeating this entire column evaluation, gradient conditions were optimized for oxamyl and aldicarb sulfone on only the Rad Pak column. The final gradient chosen for use with all eight target analytes was a 20-minute linear gradient from 80% water, 4% acetonitrile, 16% methanol to 30% water, 14% acetonitrile, 56% methanol. Figure 5 gives a typical chromatogram obtained with this column under this gradient. This is a pseudo ternary gradient which can be performed using two pumps. Pump A is set to pump pure water and Pump B is set to pump a 20:80 mixture of acetonitrile/methanol. Sample throughput is moderately high, samples can be analyzed every 25 to 30 minutes depending upon how long the column is reequilibrated at the initial conditions.

Electro Chemical Detector The HPLC conditions permit separation of the intact analytes (3). There were no problems with pyrolytic cleavage reactions in the injector as there were when the carbamoyloximes were injected into a gas chromatograph (4). After elution, the intact analytes are hydrolyzed with sodium hydroxide to release methylamine which reacts with OPA in the presence of 2-mercaptoethanol to form a highly fluorescent N-methyl-isoindole. This isoindole is also electrochemically active. A series of experiments were performed to evaluate the utility of electrochemical detection in lieu of fluorescence detection for these eight target analytes.

The effluent from the fluorescence detector was directed into an electrochemical cell. A hydrovoltamogram for aldicarb sulfone was obtained using replicate injections of 135 ng of the sulfone. The potential applied to the electrochemical cell was varied between +0.1V to +0.4V versus a standard Ag/AgCl reference electrode. The relative response of the electrochemical cell (100 nA full scale) versus the fluorescence detector (1.0 µA full scale) was plotted to give the hydrovoltamogram depicted in Figure 6. The relative response begins to flatten just above +0.4V. Subsequent experiments directed at establishing the utility of the electrochemical detector at 1 to 5 ng of sulfone per injection proved fruitless. When the sensitivity of the electrochemical detector was increased to 10 nA full scale, the background current was unacceptably high. The sulfhydryl group of 2-mercaptoethanol is also electrochemically active. Even though the applied potential was 0.4 volts less than the optimum for the sulfhydryl group, there was enough of the 2-mercaptoethanol present to cause an unacceptable background current problem when the sensitivity was increased to 10 nA full scale. Electrochemical detection has been successfully used for the OPA derivatives obtained from precolumn derivitization of amino acids (5). In this type of procedure, the excess mercaptan elutes in the void volume and is not present when the aminoacid-OPA products elute. If precolumn derivatization techniques were applied for the analysis of these eight analytes, Objective 1 could not be achieved. All eight analytes form the identical OPA derivative, and would give but a single peak using either electrochemical or fluorescence detection.

Sample Preparation Objective 3 calls for minimal sample preparation. More than 800 samples were expected during the 5-month sampling phase of this monitoring study. Samples would be received at the laboratory once every one to two weeks. Rather than using the technique typical of a GC analysis, extracting of the aqueous sample with an organic solvent, concentrating to 1 mL, and injecting 3 to 5 µL of the final extract - the alternative approach of injecting a large quantity of the aqueous sample was examined. Due to the high concentration of water in the mobile phase at the initial gradient conditions (80%) it was possible to maintain good peak shape and resolution when the injection volume was varied between 10 to 400 µL of the aqueous sample.

Sample preparation consisted of filtering (0.45 µm) the aqueous sample into an autosampler bottle. Since only 40 to 50 mL of sample were needed, sampling and transportation costs for the analysis are significantly lower than if 1 liter of sample were needed. This technique demands using a guard column before the analytical column. Without a guard column, HPLC column inlet frits block up after 80 to 100 injections. Large injections of methanolic standards (>25 µL) result in degraded resolution of aldicarb sulfone and aldicarb sulfoxide. Large injections of acetonitrile standards result in degraded resolution of aldicarb sulfone and oxamyl. These problems are circumvented by using only standards in water solutions and always injecting a constant volume anywhere between 200 to 400 µL.

Method Detection Limits The Method Detection Limits (MDLs) were established using the iterative approach (6) and the results are summarized in Table 2. During the first iteration of the MDL procedure, the injection volume was 200 µL and the spike value was a nominal 10 µg/L. The MDLs ranged from 1.04 µg/L to 4.05 µg/L with a grand average of 2.10 µg/L for the eight analytes. During the second iteration of the MDL procedure, the nominal spike level was 2.5 µg/L (approximately the average value from the first iteration) and the injection volume was 400 µL. These MDLs ranged from 0.66 µg/L to 1.57 µg/L with a grand average of 0.96 µg/L. The final value for the MDL for aldicarb sulfoxide, methomyl, and aldicarb was based on the pooled standard deviation from both iterations of the MDL procedure. In each case, these three analytes gave an F-ratio less than the critical value for the F-ratio test. The MDL values for the remaining five analytes are based on the standard deviation observed during the second iteration. Each of these five analytes gave significantly different standard deviations during the second iteration. The F-ratios were all larger than the critical value for the F-ratio test. In either case, using 200 µL or 400 µL injections permits achieving Objective 2.

Sample Stability Studies Sample stability was studied over short-term and long-term conditions to identify the important preservation techniques necessary to maintain sample integrity. The time periods studied represented the time samples would be in transit to the laboratory, the time samples sat in the autosampler tray, and the time samples would be held in the lab prior to analysis. The short-term studies involved three matrices; reagent water, raw source water, and tap water.

When dosed reagent water was held at room temperature and at neutral pH, there was a severe problem with the stability of oxamyl, 3-hydroxycarbofuran, and carbaryl. Less than 25% of each of these three analytes remained after 12 hours in unpreserved reagent water (Table 3). Upon hydrolysis, carbaryl gives 1-napththol which chromatographs separately from carbaryl and fluoresces under the conditions used in this procedure (Figure 5). Of the remaining five analytes, only methomyl and aldicarb sulfoxide exhibited greater than 75% recovery after 12 hours in unpreserved reagent water. This can lead to serious problems in the short term that samples would be in transit or be held in an autosampler tray prior to analysis. Samples

preserved at room temperature and at a pH of 3(HCl) gave recoveries quite uniform and acceptable after 12 hours. All eight analytes exhibited recoveries greater than 85% after 12 hours storage in reagent water under these conditions. Samples preserved at a pH of 4(HCl) gave results similar to unpreserved samples. With hydrochloric acid a pH of 3 was necessary to stabilize the samples.

Dosed neutral tap water (1.4 ppm free chlorine) gave short term stabilities similar to neutral reagent water. Again, there was a severe problem with oxamyl, 3-hydroxycarbofuran, and carbaryl after 24 hours (Table 4). In this series of short-term stability studies, the analytes were dosed individually rather than as a mixture of all eight analytes in order to identify oxidation or reduction products which might coelute with the other analytes. Aldicarb gave an immediate quantitative conversion to aldicarb sulfoxide when added to the unpreserved tap water. No other analyte exhibited this type of reaction. The addition of excess sodium thiosulfate destroys the free chlorine and stops these types of redox reactions. No detrimental effects due to excess sodium thiosulfate were observed when the analytes were individually dosed into reagent water containing excess sodium thiosulfate. Aldicarb was observed intact. The sodium thiosulfate did not reduce either aldicarb sulfoxide to aldicarb nor aldicarb sulfone to aldicarb sulfoxide after 12 hours storage at room temperature. The severe stability problems for oxamyl, 3-hydroxycarbofuran, and carbaryl were again evident in reagent water containing excess sodium thiosulfate.

In a neutral raw source water, the same general short-term stability problems were evident for four of the eight analytes (Table 5). Oxamyl exhibited severe stability problems which were overcome by preserving the sample at a pH of 3. Raising the pH to 11 demonstrates how facile the analytes are towards base catalyzed hydrolysis. None of the four were recovered after 2 hours at a pH of 11.

Adjusting the pH of the samples is a viable technique for maintaining sample integrity, but is a technique more suited for application in the laboratory rather than in the field. Due to transportation regulations, it may be difficult to ship samples which are known and declared to be at a pH of 3. The effects of storing samples at reduced temperatures were also examined in both the raw source water and reagent water. Again, the pH was varied between neutral and a pH of 3. The results of this long term stability study are summarized in Table 6 and indicate that cooling the samples to 5°C is an extremely useful technique to maintain sample stability - especially applicable during the time samples are in transit to the lab. After 5 days storage at 5°C, analyses indicated that the oxamyl recovery was 72% in the neutral raw source water and 96% in the neutral reagent water. Once received in the laboratory, samples targeted for oxamyl analysis should be preserved by adjusting the pH to 3 and stored at 5°C if analysis cannot be performed immediately. Matrix effects are evident in the recoveries demonstrated after 68 days storage. At neutral conditions, the raw source water exhibited uniformly lower recoveries for all four analytes when

compared to neutral reagent water. At a pH of 3, the raw source water gave higher recoveries for aldicarb sulfoxide and aldicarb sulfone whereas the recoveries for oxamyl and methomyl were nearly the same in both reagent water and raw source water.

The data in Table 6 indicate that storage stability is dependent upon the analyte, the matrix, the pH of the matrix, and the temperature of the sample. Conditions which maintain sample integrity for one analyte in a given matrix do not necessarily guarantee the integrity of another analyte. During the course of the monitoring study, the storage stability of aldicarb and its oxidation products is being studied as a function of temperature, pH, and concentration of calcium, zinc, magnesium, copper and iron at 90 different wells. These carbamates can coordinate to metals as bidentate ligands, the aldicarb sulfoxide and sulfone could coordinate to metals as tridentate ligands. The short term studies at neutral pH and the long-term study at a pH of 3 seem to indicate that aldicarb, its sulfoxide and sulfone are more stable in the raw source water than in the reagent water which contains virtually no metal ions. The results of this intensive study will be reported at a later date.

Second Laboratory Validation

The Pesticide Research Laboratory of the University of Florida performed the analyses during the monitoring study. As part of the Quality Assurance/ Quality Control (QA/QC) requirement of the final procedure (EPA Method 531), laboratories must demonstrate that the entire analytical procedure is in control by measuring analyte recoveries from relevant matrices. The frequency for this QA/QC check is once per 20 samples. This laboratory used 200 µL injections of the aqueous samples due to the equipment limitations of their refrigerated autosampler. Fifty-one samples were spiked at 5.0 µg/L each with aldicarb sulfoxide, aldicarb sulfone, and aldicarb. Dosed samples were stored at 0°C for a period of 1 to 30 days prior to analysis. Six of the samples gave greater than 150% recovery of aldicarb sulfoxide and 0% recovery of aldicarb yet gave aldicarb sulfone recoveries between 75% and 93%. This matrix effect would be expected in a chlorinated sample. The sampling teams had been instructed to sample as close to the well head as practical and prior to any point where chlorine was introduced into the water supply, however this may not have been the case with these six samples. The laboratory demonstrated that these reduced recoveries were due to a matrix effect by immediately analyzing fresh aqueous standards and demonstrating that the analytical equipment was not at fault. Two of the samples gave interference problems which precluded measuring the recovery of either aldicarb sulfoxide or aldicarb and one sample gave significantly reduced recovery ($\alpha = 0.005$) for aldicarb sulfone.

A statistical summary of the dosed matrix data gives the following. Aldicarb sulfoxide exhibited an average recovery of 85.4% ± 14.9% with a relative standard deviation (RSD) of 17.5% in 44 samples. Aldicarb sulfone gave an average recovery of 82.8% ± 20.5% (RSD = 24.8%) in 50 samples and aldicarb gave an average recovery of 90.2% ± 10.8% (RSD = 11.9%) in 44

samples. Matrix effects (α = 0.005) were noted in 7 of 51 samples (14%) for aldicarb sulfoxide and aldicarb, six of these samples were suspected of containing residual chlorine, and in 1 of 51 samples (2%) for aldicarb sulfone.

CONCLUSIONS

Aldicarb and seven other carbamate and carbamoyloxime pesticides can be analyzed efficiently and selectively at low µg/L levels using the techniques of direct aqueous injection HPLC followed by post column derivatization with o-phthalaldehyde. Preservation of samples at 5°C and/or at a pH of 3 is necessary to maintain sample integrity. The overall procedure achieves the objectives desired. The sample preparation is minimal, the analysis is very amenable to unattended operation, and aldicarb, aldicarb sulfoxide and aldicarb sulfone gave MDLs of 1.3, 0.8, and 0.5 µg/L, respectively.

Electrochemical detection of the post column OPA derivatives is not a viable alternative to fluoresence detection due to the presence of excess 2-mercaptoethanol. Electrochemical detection techniques might be useful if the excess mercaptan were eliminated prior to the detection--possibly by adding another mixing tee and reacting the mercaptan with bromoacetic acid just prior to the electrochemical cell. Precolumn derivatization techniques would allow successful electrochemical detection--but would only permit measurement of total carbamates and carbamoxyloximes. This might be a useful screening procedure but is not a useful procedure for the individual carbamates and carbamoyloximes.

A very recent description of a virtually identical procedure (7) agrees well with most of the observations concerning sample stability. Dramatic losses for oxamyl, 3-hydroxycarbofuran, and carbaryl were found when samples at a pH of 8.3 were stored at ambient conditions for 19 hours. No losses were observed when samples were adjusted to a pH <6 with orthophosphoric acid and held at ambient conditions for 19 hours. Orthophosphoric acid may be a better preservative for these analytes than hydrochloric acid since the addition of hydrochloric acid did not exhibit any preservative effects at a pH of 4 but did at a pH of 3.

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Table 1. Physical Description of HPLC Columns Evaluated

Column	Particle size, (μm)	ID (mm)	Length (cm)	Flow Rate (mL/min)
μBondapak C-18	10	3.9	30	1.0
μBondapak CN	10	3.9	30	1.0
μBondapak Phenyl	10	3.9	30	1.0
Rad Pak C-8	10	5.0	10	1.0
Rad Pak C-18	10	5.0	10	1.5
Rad Pak PAH	10	5.0	10	1.5
Rad Pak C-18	10	8.0	10	2.0
Supelcosil LC-PAH	5	4.6	25	1.0
Supelcosil LC-8	5	4.6	25	1.0
LiChrosorb RP-2	5	4.6	25	1.0
Zorbax TMS	6	4.6	25	1.0

Table 2. Method Detection Limits Studies

Analyte	Spike Level[a] (µg/L)	Mean Found (µg/L)	Percent Recovery	Std. Dev. (µg/L)	MDL[b] (µg/L)	FC Ratio	MDL (µg/L)
Aldicarb sulfoxide	10.4	10.3	99	0.34	1.07		
	2.40	2.4	100	0.22	0.69	2.39	0.8[d]
Aldicarb sulfone	10.2	11.1	109	0.52	1.63		
	2.56	2.61	102	0.16	0.50	10.5	0.5
Oxamyl	10.2	14.1	138	1.29	4.05		
	2.91	2.65	91	0.50	1.57	6.66	1.6
Methomyl	10.5	10.1	96	0.33	1.04		
	2.78	2.47	89	0.21	0.66	2.47	0.7[d]
3-Hydroxy-carbofuran	10.1	10.7	106	0.97	3.05		
	2.41	2.39	99	0.50	1.57	3.76	1.6
Aldicarb	10.2	10.3	101	0.60	1.89		
	2.55	2.85	112	0.35	1.10	2.94	1.3[d]
Carbofuran	10.7	11.4	112	0.99	3.11		
	3.20	2.81	88	0.28	0.88	12.5	0.9
Carbaryl	10.3	12.5	123	0.50	1.57		
	2.57	1.98	77	0.22	0.69	5.16	0.7

a Seven replicates at 10 µg/L with 200 µL injections; seven replicates at 2.5 µg/L with 400 µL injections.
b MDL = 3.143 * standard deviation.
c Critical F-ratio is 3.05.
d Pooled MDL used; if F ratio < F-critical, MDL pooled = pooled standard deviation * 2.681.

Table 3. Short-Term Stability Data for Reagent Water Held at Room Temperature

Analyte	Dose Level (µg/L)[a]	Reagent Water Recovery pH = 7 (time in hours) 0	6	12	pH = 3 (time in hours) 0	6	12
Aldicarb sulfoxide	1000	100	81	77	100	96	100
Aldicarb sulfone	1000	100	70	56	100	96	97
Oxamyl	1000	100	16	5	100	96	94
Methomyl	1000	100	88	90	100	94	93
3-Hydroxycarbofuran	1000	100	33	16	100	97	95
Aldicarb	1000	100	94	33	100	93	96
Carbofuran	1000	100	60	55	100	96	87
Carbaryl	1000	100	26	9	100	93	96

[a]Analysis of 20 µL injections of aqueous samples.

Table 4. Short-Term Stability Data for Tap Water and Sodium Thiosulfate Preserved Reagent Water

Analyte	Dose Level (µg/L)	Tap Water Recovery[a] 3[c]	Tap Water Recovery[a] 24[c]	Sodium Thiosulfate Preserved Reagent Water[b] 3[c]	Sodium Thiosulfate Preserved Reagent Water[b] 24[c]
Aldicarb sulfoxide	1000	98	70	98	76
Aldicarb sulfone	1000	87	52	94	60
Oxamyl	1000	75	9	76	5
Methomyl	1000	97	84	96	90
3-Hydroxycarbofuran	1000	91	26	94	12
Aldicarb	1000	d	e	100	84
Carbofuran	1000	103	66	89	30
Carbaryl	1000	94	21	62	2

a 1.4 ppm free chlorine.
b 2.5 grams sodium thiosulfate per liter.
c time in hours.
d 116% as aldicarb sulfoxide.
e 98% as aldicarb sulfoxide.

Table 5. Short-Term Stability Data for Raw Source Water Held at Room Temperature

Analyte	Dose Level (µg/L)	Raw Source Water Recovery[a] pH = 7 2[b]	pH = 7 30[b]	pH = 7 96[b]	pH = 3 96[b]	pH = 11 2[b]
Aldicarb sulfoxide	640	98 ± 6	96 ± 10	83 ± 4	91 ± 4	ND[c]
Aldicarb sulfone	420	92 ± 7	89 ± 4	74 ± 7	105 ± 3	ND
Oxamyl	1050	95 ± 4	18 ± 1	ND	119 ± 3	ND
Methomyl	4909	100	100	100	100	ND

a Four replicates, methomyl treated as an internal standard.
b Time in hours.
c ND = not detected.

Table 6. Long-Term Stability Data for Reagent Water and Raw Source Water Held at 5°C

Analyte	Dose Level µg/L	Reagent Water Recovery pH=7 time in days 5[a]	pH=7 time in days 68[b]	pH=3 time in days 5[a]	pH=3 time in days 68[b]	Raw Source Water Recovery pH=7 time in days 5[a]	pH=7 time in days 68[c]	pH=3 time in days 5[a]	pH=3 time in days 68[a]
Aldicarb sulfoxide	640	100 ± 4	100 ± 3	95 ± 1	45 ± 4	98 ± 2	67 ± 2	98 ± 2	70 ± 3
Aldicarb sulfone	420	97 ± 5	103 ± 4	102 ± 3	71 ± 3	100 ± 3	36 ± 3	103 ± 3	91 ± 4
Oxamyl	1050	96 ± 2	80 ± 12	109 ± 4	91 ± 9	72 ± 3	ND	111 ± 5	96 ± 4
Methomyl	490	100[d]	92 ± 2	100[d]	83 ± 7	100[d]	78 ± 2	100[d]	95 ± 5

[a] Four replicates.
[b] Six replicates.
[c] Five replicates.
[d] Methomyl treated as internal standard for 5 day data.
ND - Not detected.

Table 7. Acceptable Storage Time for Selected Method 531 Analytes[a]

Analyte	Reagent Water Recovery Time in Days pH = 7	Reagent Water Recovery Time in Days pH = 3	Raw Source Water Recovery Time in Days pH = 7	Raw Source Water Recovery Time in Days pH = 3
Aldicarb Sulfoxide	70	18	35	40
Aldicarb Sulfone	70	41	19	70
Oxamyl	53	65	3	70
Methomyl	70	62	70	70

[a] All samples stored at 5°C. Acceptable indicates time to show a 15% loss of analyte.

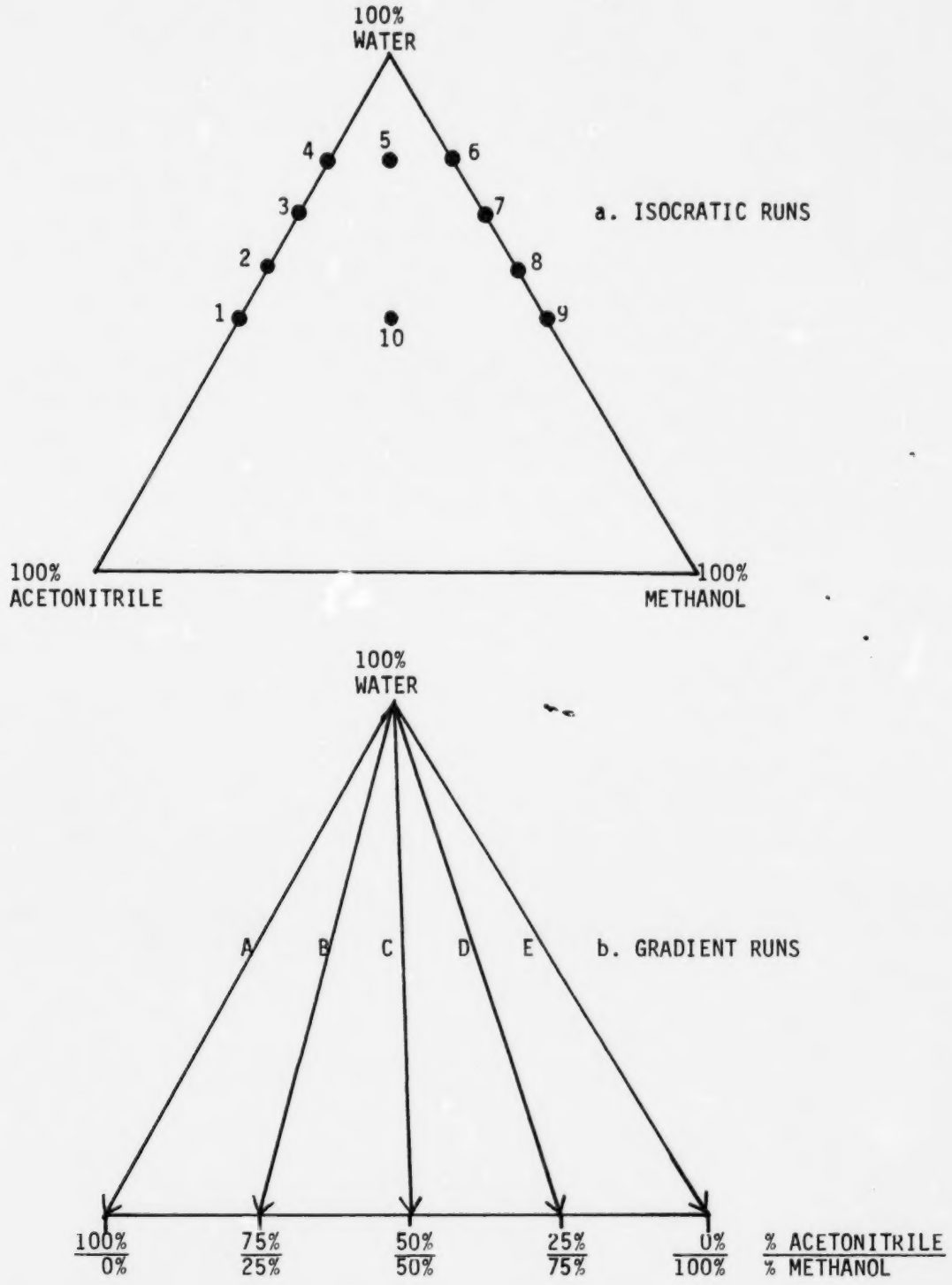

Figure 1. Ternary Diagrams Summarizing Chromatographic Experiments

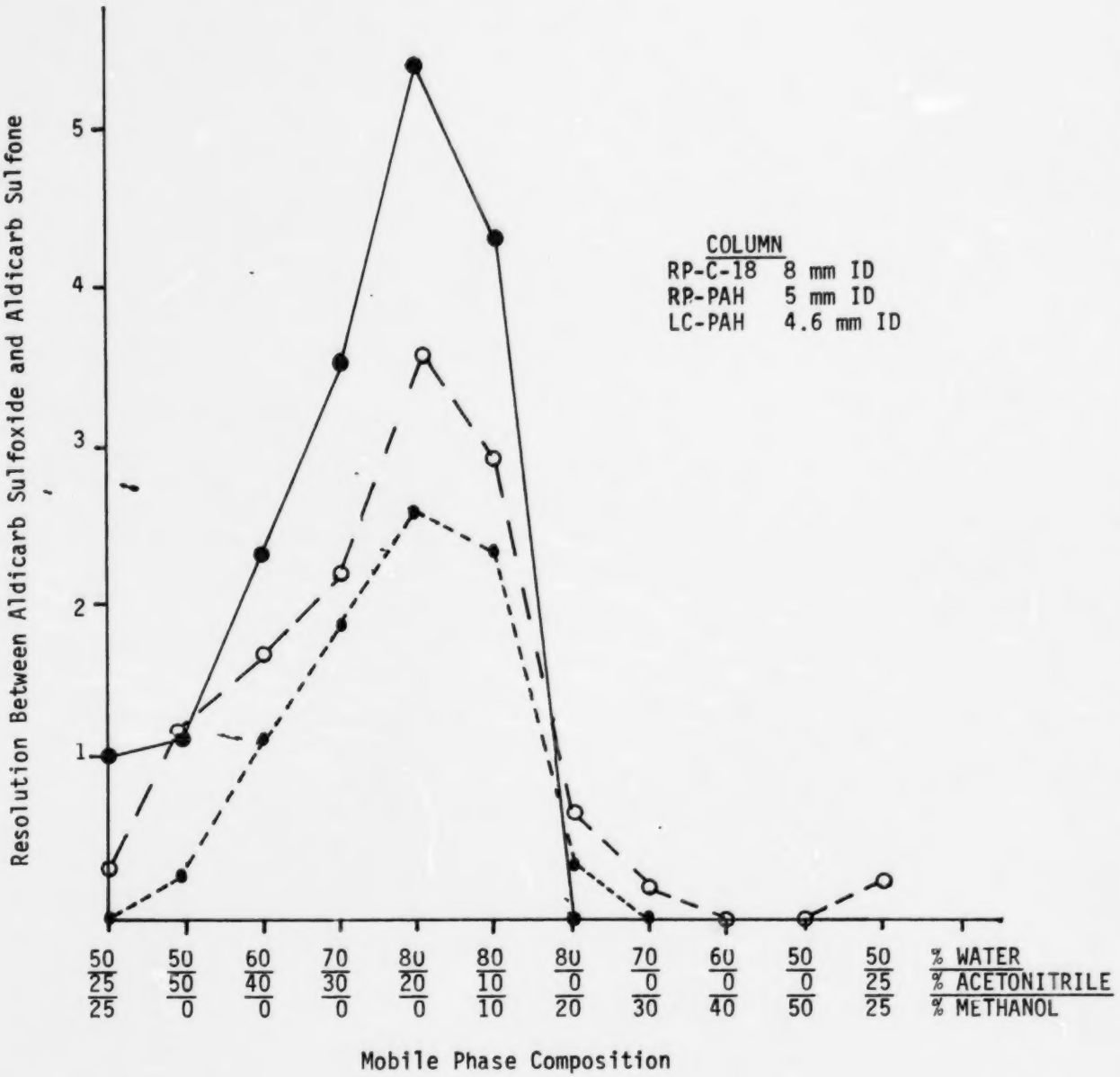

Figure 2. Resolution Between Aldicarb Sulfoxide and Aldicarb Sulfone Versus Isocratic Mobile Phase Composition

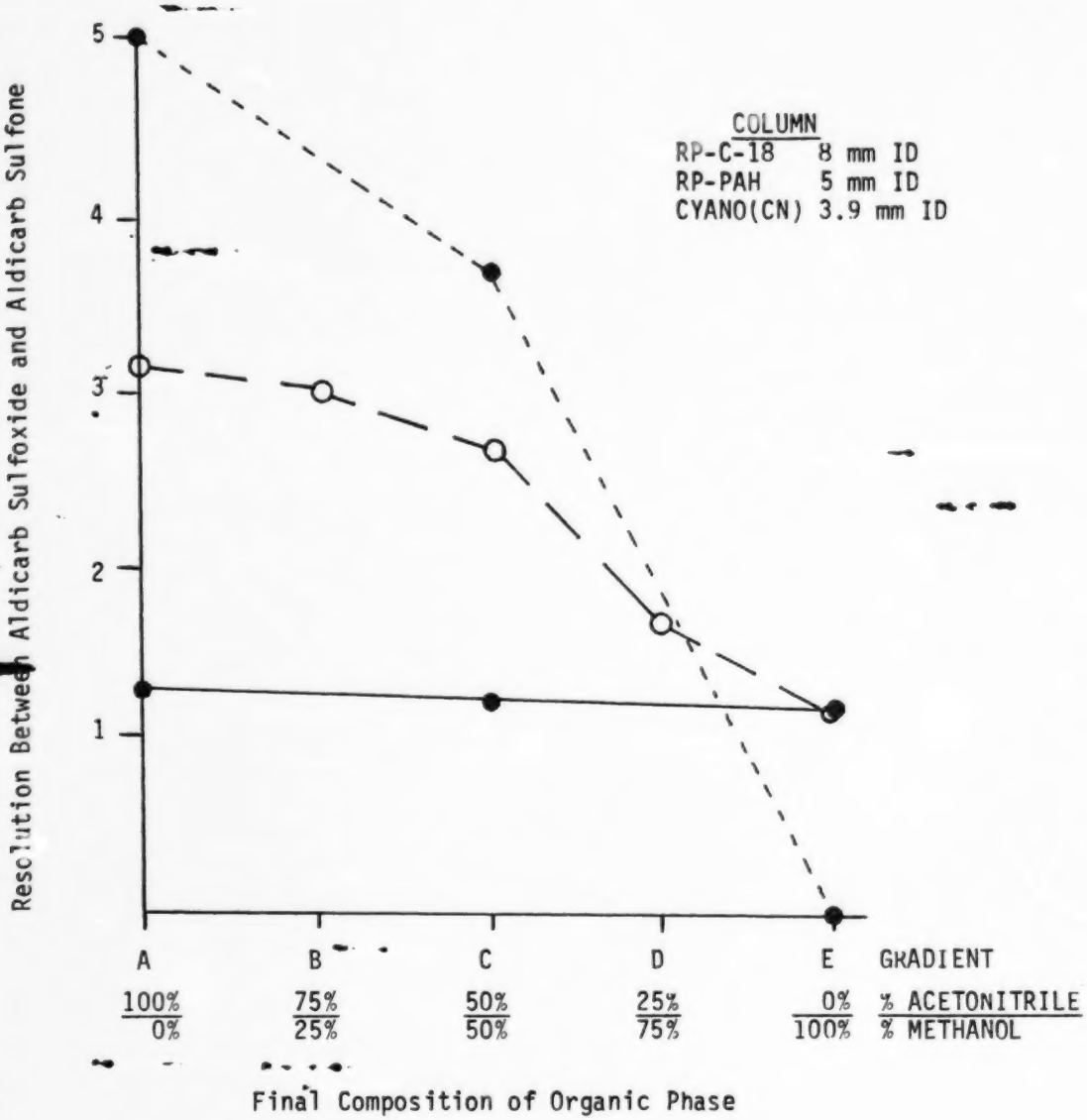

Figure 3. Resolution Between Aldicarb Sulfoxide and Aldicarb Sulfone Versus Final Composition of Gradient

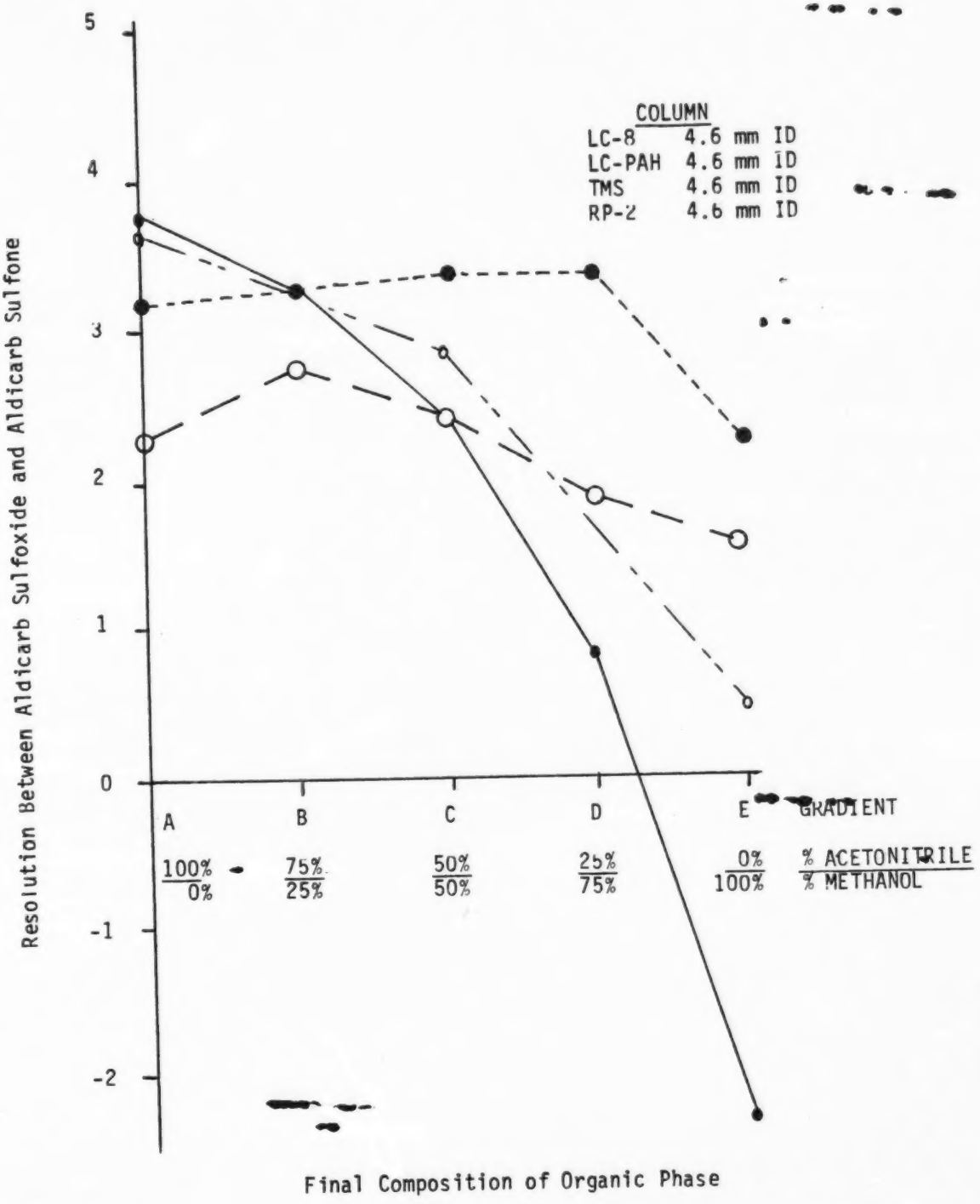

Figure 4. Resolution Between Aldicarb Sulfoxide and Aldicarb Sulfone Versus Final Composition of Gradient

COLUMN 8 mm ID x 10 cm Rad-Pak C-18
GRADIENT from 80%:4%:16% (Water:Acetonitrile:Methanol)
to 30%:14%:56% (W:A:M) in 20 minutes (linear)
DETECTOR Fluorescence; 235 nm/ex, >419 nm/em

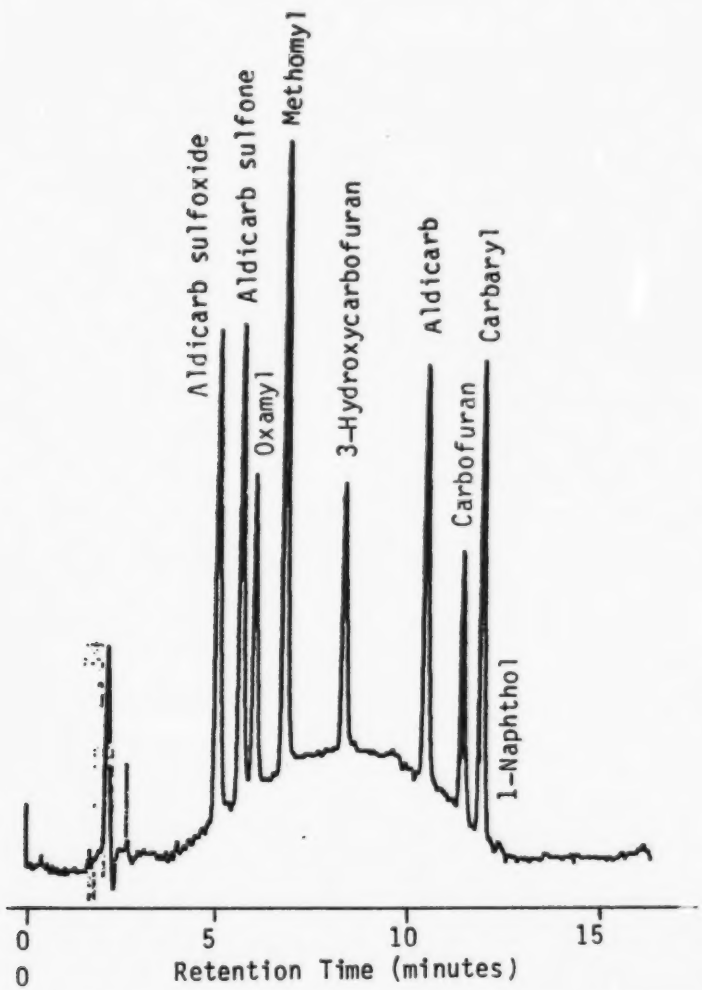

Figure 5. HPLC Chromatogram of N-Methyl Carbamoyloximes and N-Methyl Carbamates

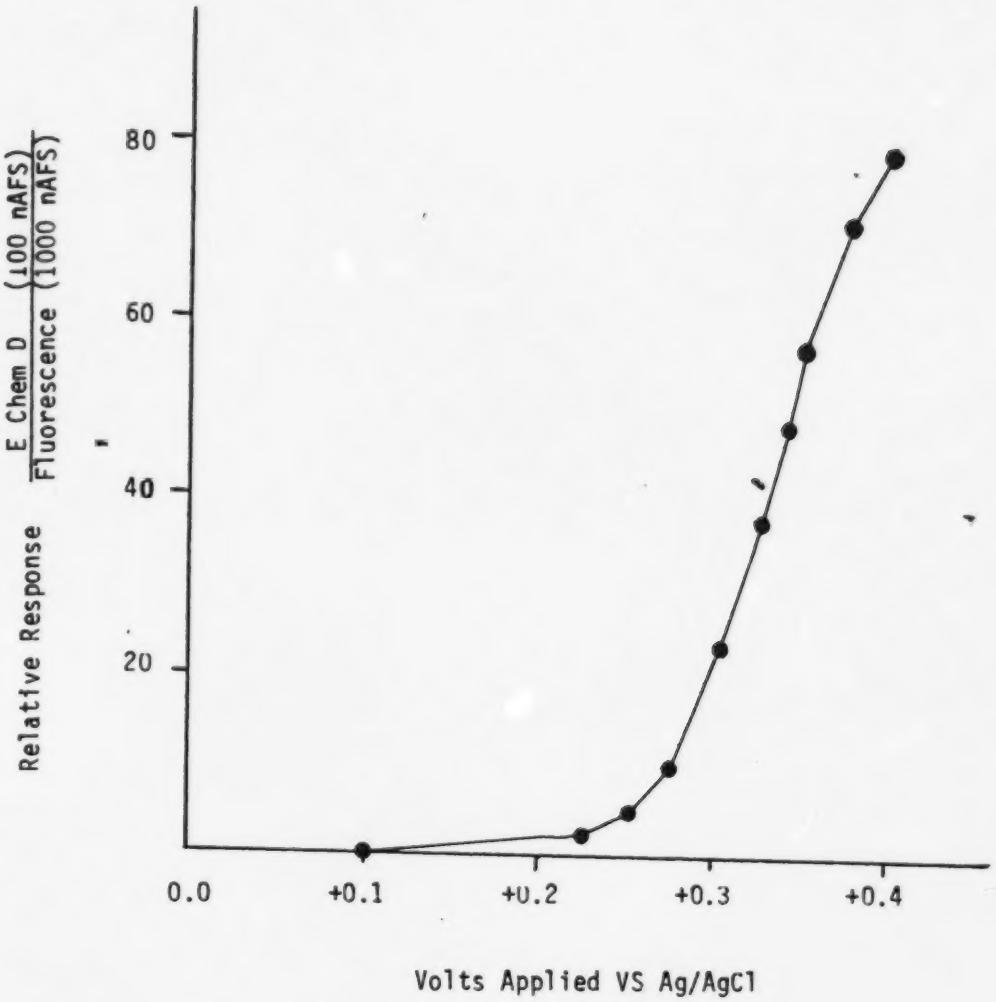

Figure 6. Hydrovoltamogram of OPA Derivative of Aldicarb Sulfone